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A field investigation of short-range dispersal by female *Simulium damnosum* s.l.

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ABSTRACT

Female *Simulium damnosum* s.l. were caught at a site in a savannah zone beside the Mono River in Togo and at varying distances westwards perpendicular to it in an experiment to investigate short-range dispersal by the flies. The numbers of flies caught and the percentages that were parous decreased with increasing distance from the river, the latter suggesting that nulliparous flies disperse on average further than parous flies. The decreases were quantified using statistically significant multiple regressions involving distances from the river and the days since the start of the experiment because there was much day-to-day variation, probably attributable to the flies' gonotrophic cycles. For future modelling purposes a relationship between numbers caught and distance alone was also estimated for both numbers caught and parous rates. Of the different members of the *S. damnosum* species complex identified in larval samples, *S. damnosum* s.str. predominated (66.7%), with *S. squamosum* accounting for 25.5% and the Beffa form of *S. soubrense* for 7.8%, proportions that were not significantly different from those of adults identified at the river and 10 km away. A small sub-sample of dissected parous flies showed that transmission was occurring at the riverside and at 10 km away from the river.

1. Introduction

Female *Simulium damnosum* s.l. Theobald 1903 (Diptera: Simuliidae), the vectors of onchocerciasis in West Africa, are known to travel very long distances of up to 500 km during wind-assisted migrations (Garms et al., 1979; Walsh et al., 1981; Cheke and Garms, 1983; Baker et al., 1990). They also disperse up to 40 km away from breeding sites on appetitive flights in search of blood meals (Crisp, 1956; Le Berre, 1966; Duke, 1975; Renz and Wenk, 1987). Although Crosskey (1956) pointed out that villages in northern Nigeria up to 15 km away from rivers could be subjected to high levels of onchocerciasis transmission, this aspect of blackfly behaviour is poorly researched. Most onchocerciasis transmission data are obtained from entomological studies at riversides, but transmission also occurs away from the rivers' banks. Thus, knowledge of short-range dispersal of the vectors is of importance to inform decisions on which populations require chemotherapeutic treatments and how far away from the rivers such treatments will be necessary. Delimiting the target population for mass drug administration, or which river sections to treat with insecticides, are important considerations as control operations move towards the goal of onchocerciasis elimination as envisaged by the WHO roadmap (WHO, 2021). Another important question is how extensive are transmission zones? These are defined as geographical areas where transmission of *Onchocerca volvulus* Leuckhart 1893 occurs by locally breeding vectors, and which can be regarded as natural ecological and epidemiological units for interventions (WHO, 2016). Transmission zones are increasingly being used to define areas needing control activities and thus it becomes necessary to know how far away from centres of blackfly breeding do the flies continue to transmit onchocerciasis.

It has been reported that the numbers and parous rates of savannah blackfly species, such as *S. damnosum* s.str. Vajime and Dunbar, 1975 and *S. sirbanum* Vajime and Dunbar, 1975, decrease with increasing distance from breeding sites (Le Berre, 1966; Duke, 1975; Renz and Wenk, 1987), suggestive of lower prevalence and intensity of infections in villages with increased distance from blackfly breeding sites. However, De Sole et al. (1991) found that where *S. sirbanum* was the main vector, only one (Milo focus, Guinea) out of three study zones had this pattern whilst two others (Tienfala on the River Niger in Mali and the River Gambia foci in Senegal) were quite different, with the former reporting the highest intensity of infection over 10 km from blackfly breeding sites. In a forest zone, Duke (1975) also found blackflies away from a river's bank tended to be older and included infective flies. In

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Table 1
Numbers of larvae of different members of the *S. damnosum* species complex collected in July-August 1984 in the Mono river at Landa-Mono and identified by cytotaxonomy shortly before and during the experiment.

Numbers of larvae of the *S. damnosum* complex				
Date of collection	*S. damnosum* s.str.	*S. squamosum*	*S. soubrense* (Beffa form)	Total
26 June	14	6	3	23
10 July	23	8	5	36
26 July	38	14	3	55
3 August	27	11	1	39
Total (%)	102 (66.67)	39 (25.49)	12 (7.84)	153

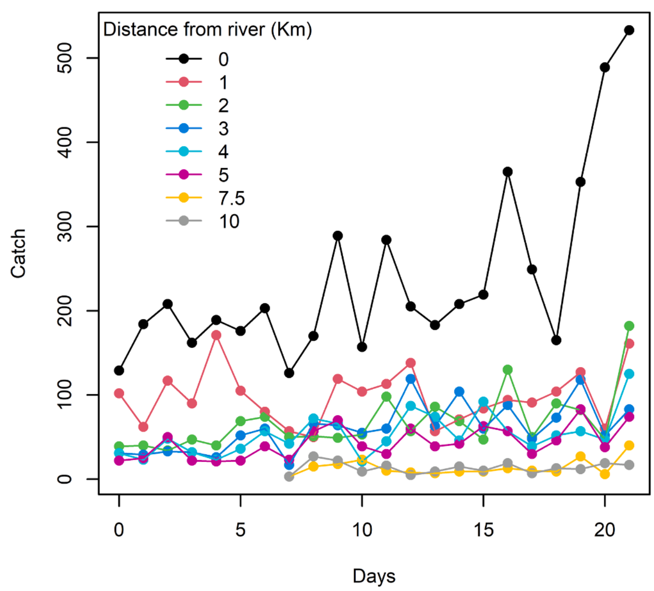

Fig. 1. The numbers of female *Simulium damnosum* s.l. caught on different days and at different distances away from Landa-Mono. The inset gives colour codes for the distances.

Sierra Leone, Bockarie and Davies (1990) found more vector-host contact in open farmland than at riversides or in villages. Given these contrasting results, we present additional results here based on an experiment conducted during the WHO Onchocerciasis Control Programme in West Africa (OCP). The experiment was designed to investigate the short-range dispersal of blackflies away from their breeding site at a savannah site in Togo. Also, it is hoped that quantitative relationships derived from analysis of the data could be of assistance to modelling exercises aimed at assisting elimination planning.

2. Materials and methods

2.1. Study site

The experiment was conducted at Landa-Mono (08° 36′N, 01° 16′E) beside the Mono river, where there was a breeding site of *S. damnosum* s. l., in July-August 1984. At the time there was a road running westwards from the river along which *S. damnosum* s.l. were caught using standardised human landing catches beginning at 0700 h and finishing at 1800 h every day (Walsh et al., 1978). Five catching teams of two people

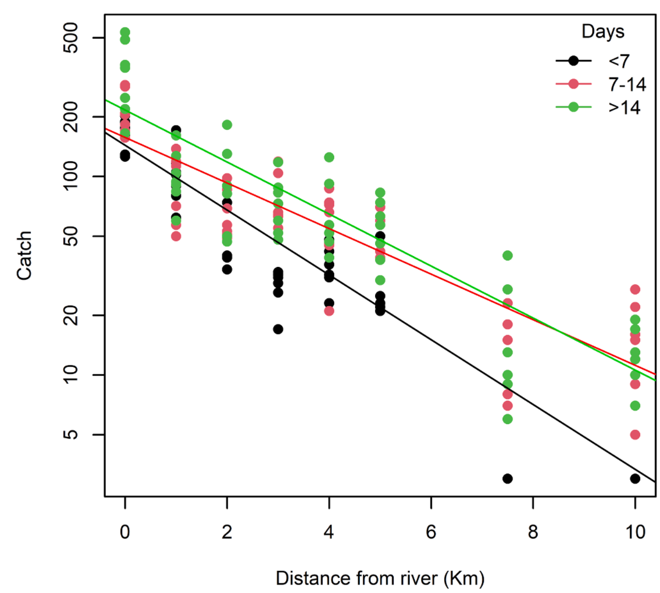

Fig. 3. Numbers of female *S. damnosum* s.l. separated into 7-day bins according to distance (km) from Landa-Mono. Inset shows colour codes for the 7-day bins. Both distance and day groups are significant overall (analysis of covariance, $P < 0.0001$ in both cases). Regression lines predicted by negative binomial GLM, for which the slopes are significantly different (analysis of covariance, $P = 0.009$).

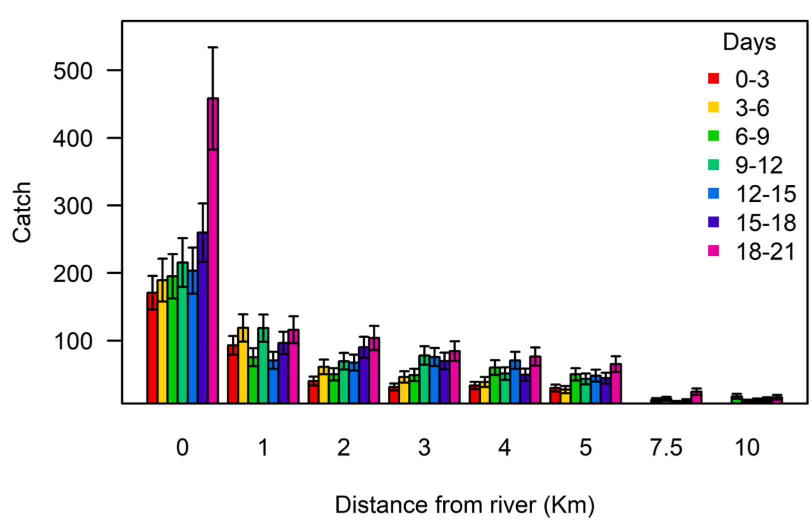

Fig. 2. Numbers of female *S. damnosum* s.l. caught at different distances (km) from Landa-Mono according to time of capture. Inset gives the colour codes for bins of different 3-day periods.

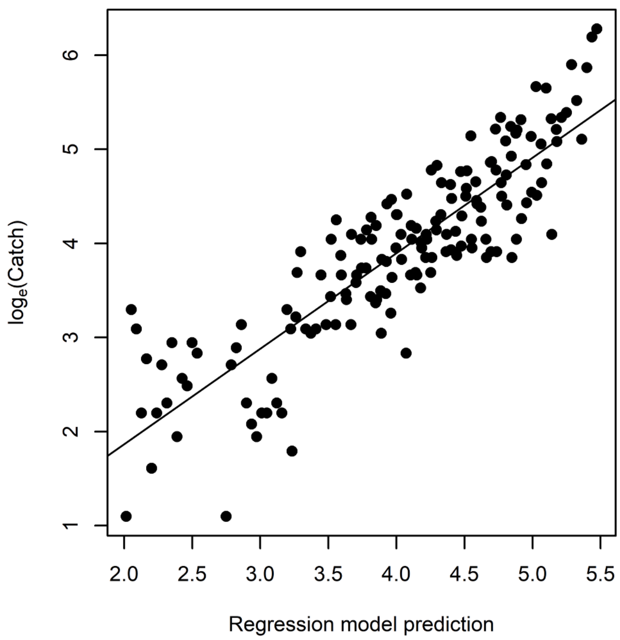

Fig. 4. Natural logarithms of the numbers of female *S. damnosum* s.l. caught as predicted by the multiple regression equation $\log_e(\text{catch}) = 4.691 + 0.0372*\text{days} - 0.2936*\text{distance}$.

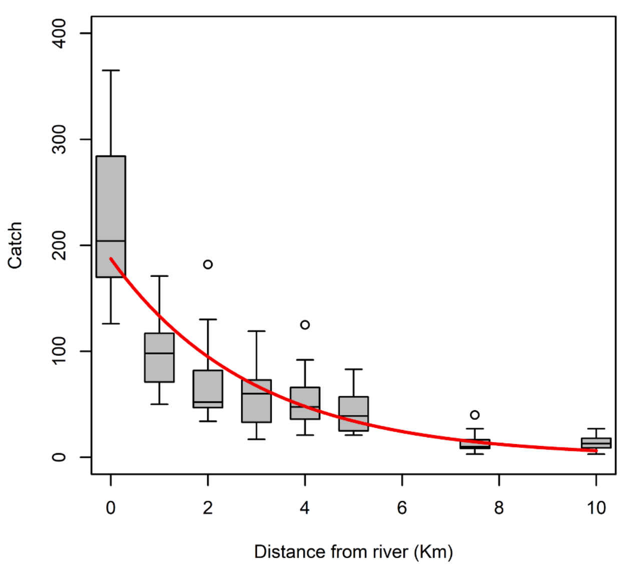

Fig. 5. Relationship between numbers of female *S. damnosum* s.l. caught and distance away from the river ($\log_e(\text{catch}) = 5.233 - 0.3399*\text{Distance}$; $P < 0.0001$, chisquare = 7050, df = 1, from Poisson GLM).

each rotated their positions to minimise biases, at various distances (0, 1, 2, 3, 4 & 5 km) away from the blackfly breeding site from 16 to 22 July, with additional sites requiring two more catching teams at 7.5 and 10 km added from 23 July until 6 August. The site at Landa-Mono no longer exists as the area was flooded by the upstream construction of the Nangbeto dam, which was commissioned in 1987.

2.2. *Identifications and ageing of larval and adult blackflies*

Mature larvae were collected from breeding sites in the river at Landa-Mono and fixed in Carnoy's fluid (3 parts ethanol: 1 part glacial acetic acid). Later, after staining with Feulgen's stain, they were identified according to their salivary gland polytene chromosomes, as described by Vajime and Dunbar (1975) and Meredith et al. (1983).

Except on the last day (6 August), all (or sub-samples if catches exceeded 100 flies) of the adult flies collected were examined for physiological age and classified as parous or nulliparous, as described by Lewis (1958). All flies were preserved in 70% methanol, separated according to date and physiological age, and sub-samples were later identified so far as is possible by morphological methods using combinations of published methods (Garms, 1978; Garms et al., 1982; Garms and Cheke, 1985). Some sub-samples were further dissected in search of infections with *O. volvulus*.

Table 2
Numbers of female *S. damnosum* s.l. caught, numbers parous, percent parous at Landa-Mono and increasing distances away, plus for the latter the parous as percentage of the parous rate at Landa-Mono. Data for 16 July to 5 August excluding data for catches at 7.5 and 10 km distant from Landa-Mono and data for after the additions, 24 July to 5 August.

Distance from Landa-Mono	Numbers caught	Numbers dissected	Numbers parous	% parous	Parous as% of parous at Landa-Mono
Data for 16 July to 5 August					
0	4713	1494	915	61.2	–
1	1996	1329	523	39.4	57.2
2	1302	1035	423	41.7	47.2
3	1250	975	382	39.2	41.7
4	1047	950	425	44.7	46.4
5	878	826	380	46.0	41.5
Data for 24 July to 5 August					
0	3462	805	446	55.4	–
7.5	167	164	60	36.6	13.5
10.0	186	183	70	38.3	15.7

2.3. *Statistical methods*

The R package (R Core Team, 2022) was used for the analyses. Count data, either multiple regressions or analyses of variance with time and distance categories, were analysed using generalised linear models with log links and negative binomial errors. Analyses of percentage parous data had logit links and quasi-binomial errors (because of over-distribution). Cross correlations between collection sites used progressively lagged detrended time series data.

3. Results

Cytotaxonomic identifications of larvae collected in the Mono river at Landa-Mono shortly before and during the trial are given in Table 1. Fig. 1 shows the numbers caught at different distances away from Landa-Mono according to capture day. Unsurprisingly, the catches were always highest at the riverside. It is also apparent that there were successive peaks and troughs, probably reflecting gonotrophic cycle lengths. Time series analysis of the daily catches at Landa-Mono from 20 May to 31 August 1984, thus overlapping the period of this study, suggested that there were cycles of 2.4 days (Cheke, 1995). To take account of this, the data were re-arranged into 3-day bins, i.e. 0–3 days, 3–6 days, 6–9 days etc. and Fig. 2 shows this configuration, clearly showing how the numbers caught declined with distance away from the river. A 2-way analysis of variance confirmed that the numbers caught were related to the 3-day period and the distance with statistical significance of $P < 0.00001$ in both cases, and the interaction between the 3-day bins and distances approached statistical significance ($P = 0.055$).

The decline of the numbers caught can also be well illustrated by using 7-day bins and plotting the numbers caught against distance from the river (Fig. 3.). The regressions for the 3 lines in Fig. 3 have significantly different slopes (analysis of covariance, $P = 0.009$). The natural logarithms of the numbers caught, without separation into bins, were

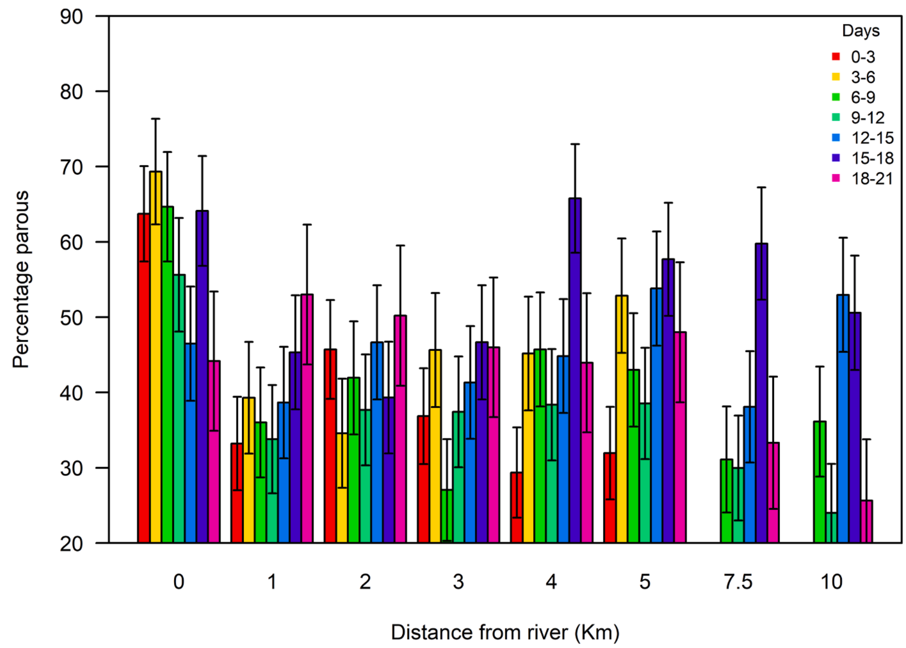

Fig. 6. Percentages of parous flies in the samples of female *S. damnosum* s.l. collected at Landa-Mono and at increasing distances from it separated into 3-day bins. Inset shows colour codes for different 3-day bins.

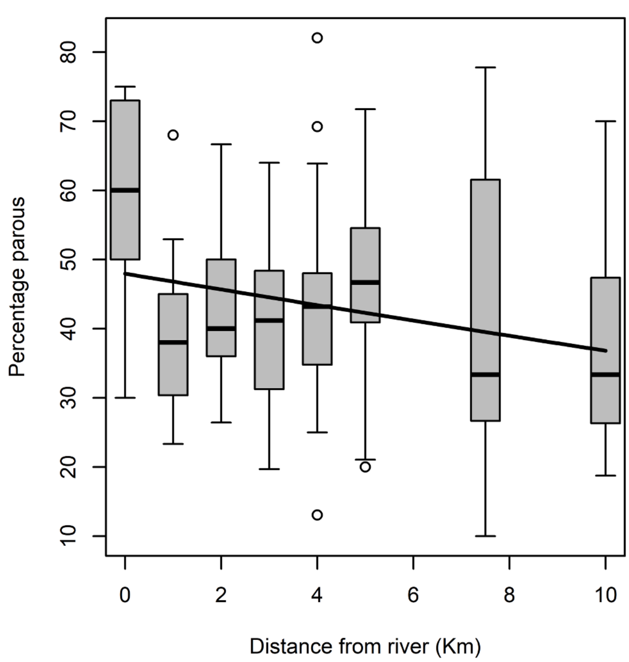

Fig. 7. Variation of percentage parous female *S. damnosum* s.l. with increasing distance (km) from the river. Percent parous = 47.91 – 1.116*distance.

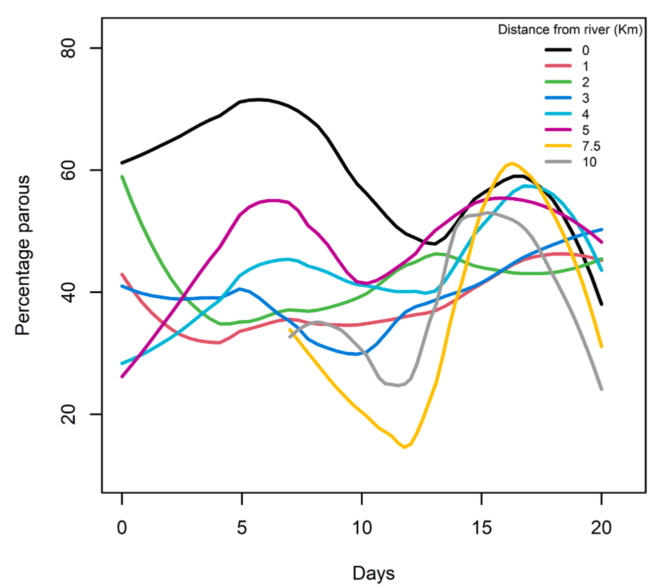

Fig. 8. Percentages parous of female *S. damnosum* s.l. caught at Landa-Mono and at different distances away from it according to the day of capture. Inset shows colour codes for distances from the river. Lines have been smoothed with locally weighted smoothing (LOESS).

shown by a multiple regression with a negative binomial adjusted general linear model to be significantly related to the day of capture ($P < 0.00001$) and distance from the river ($P < 0.00001$) according to the formula $\log_e(\text{catch}) = 4.691 + 0.0372*\text{days} - 0.2936*\text{distance}$ (Fig. 4). However, since the days component of this relationship will be irrelevant to other studies of this sort, we re-calculated the formula excluding the numbers of days which gives the highly significant relationship of $\log_e(\text{catch}) = 5.233 - 0.3399*\text{Distance}$ ($P < 0.0001$, chisquare = 7050, df = 1, from Poisson GLM; Fig. 5).

The parous rates 1 km or more from Landa-Mono were consistently lower than at the riverside, tending to decrease with increasing distance but not markedly so (Table 2), as is also illustrated when the data are separated into 3-day bins (Fig. 6). However, the effects of both distance and 3-day bin periods on the parous rates are both significant (2-way ANOVA with quasi-binomial log link, $P = 0.0003$ for 3-day periods and $P < 0.0001$ for distance). Furthermore, for the unseparated data there is a significant multiple regression estimated from a quasibinomial general linear model for the logit of the percentage parous of $\log_e$ (pcparous/(100-pcparous)) = days*0.0215 – distance*0.0541 – 0.2818 ($P = 0.000002$, df = 2151, $F = 14.54$). As with the data on numbers caught, we also calculated this relationship excluding the factor for days, giving percent parous = 47.91 – 1.116*distance ($P < 0.007$), for comparative purposes and for future modelling. This relationship implies that the percentages of parous flies decline by 1.11% per km, on average, suggesting that the nulliparous flies disperse further than the parous flies. However, visually, this relationship does not appear to be very strong up to 5 km distant (Fig. 7). The daily parous rates at Landa-Mono ranged from 30.0 to 75.0%, but 10 km away they ranged from only 18.7 to 70%. Overall, they were 55.4% at Landa-Mono but only 38.2% 10 km away, differences that are statistically significant (chi-square = 63.04, $P <$ 0.001).

Variations in the percentages parous caught with time followed similar curvilinear patterns at each distance from Landa-Mono, as illustrated by Fig. 8. This may reflect the gonotrophic cycles as the rate of production of flies is not constant and so the percentages parous will also vary, as the insects find a blood-meal and return to the river to lay their eggs, approximately every 2–4 days. Further evidence of such cycles was provided by the result that the only significant ($r = 0.45$, $P <$ 0.05, $N = 20$) cross correlation of de-trended time series (trends removed for both distance and days) for each distance was of a lag of two days to the peak correlation.

Sub-samples of the adult flies caught at Landa-Mono and 10 km away were identified using morphological methods. At Landa-Mono 68 (70.1%) were *S. damnosum* s.str./ *S. sirbanum*, 21 (21.6%) were *S. squamosum* and 8 (8.2%) were the Beffa form of *S. soubrense*. At the 10 km away site, 46 (67.6%) were *S. damnosum* s.str./ *S. sirbanum*, 20 (29.4%) were *S. squamosum* and 2 (2.9%) were the Beffa form of *S. soubrense.* These proportions were not significantly different from each other nor different from the larval samples ($P = 0.55$, chi-square = 3.07, df = 4, on a 3 × 3 chi-square contingency test).

Ninety-seven of the parous flies from Landa-Mono and 68 from the site 10 km away were dissected for the presence of larvae indistinguishable from those of *O. volvulus.* At the former, 11.3% harboured first or second stage larvae (L1/L2) and 1 fly (1.0%) was infective with a third stage larva. The data for 10 km away were very similar (11.8% with L1/L2, 1.5% infective), thus transmission was still occurring 10 km away from the breeding site. All of the infections were in flies identified as *S. damnosum* s.str. / *S. sirbanum*, with the exceptions of one Beffa form of *S. soubrense* fly at Landa-Mono that harboured 6 L1 larvae and one *S. squamosum* fly caught 10 km west of Landa-Mono that had 1 L1 larva.

4. Discussion

The results showing decreasing numbers and decreasing parous rates with increasing distances from the riverside are consistent with previous studies in savannah zones (Le Berre, 1966; Duke, 1975; Renz and Wenk, 1987). There were no *S. sirbanum* found in our study, but this species was involved in two of the studies with contrasting results (De Sole et al., 1991) discussed in the Introduction, so the cytospecies involved may determine the behaviour found. The results from other contrasting studies (Duke, 1975; Bockarie and Davies, 1990) could be accounted for by differing habitats as well as the presence of different vector taxa.

This study has not only confirmed previously described trends in savannah areas of West Africa but has also provided quantitative relationships that it is hoped will assist modelling of how best to deploy resources in control operations in the future. Our study was restricted to monitoring westward movements, partly because to the east of the river there was a densely wooded area that was uninhabited for about 10 km and inaccessible when the river waters were high. It is possible that the flies' dispersal patterns could be different in directions other than westward and be affected by strong winds, especially those associated with line squalls. Therefore, additional extensive field work, perhaps repeated in different habitats and with a variety of vector taxa and examining radial dispersal in different directions, is needed to improve understanding of the dispersal of *S. damnosum* s.l. from its breeding sites to assist with planning control interventions. It is also hoped that the methods used to analyse the data described here, taking account of daily variations and gonotrophic cycle lengths, could be useful for analysing future investigations of this sort.

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Ethical approval

The research was approved as part of the activities of the WHO Onchocerciasis Control Programme.

CRediT authorship contribution statement

J. Frank Walsh: Conceptualization, Investigation, Writing – review & editing, Funding acquisition. **Robert A. Cheke:** Investigation, Writing – original draft, Writing – review & editing. **Stephen Young:** Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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